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Comparative study of lipid profile in young smokers and non smokers

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ABSTRACT

A prospective study was carried to find the percentage of dyslipidemia among smokers in comparison with non smokers to study the alteration of lipid profile among smokers and non smokers. To see any dose related changes 50 male smokers and 50 male non smokers of Karimnagar District were included in this study. Serum lipid profile was analysed in all the subjects. The labeling of dyslipidemia was done according to NCEP guide lines. Dyslipidemia was present among 88% of smokers, with HDL dyslipidemia (HDL < 40 mg/dL) in 78%, LDL dyslipidemia (LDL > 130 mg/dL) in 58%, and Hyper tryglideridemia (TG > 150 mg/dL) in 58% and Total cholesterol (TC > 200 mg/dL) in50%. Dyslipidemia among smokers indicates greater risk of atherogenic disorder, which may be higher as the number of cigarettes and duration of smoking increases.

Keywords: Lipid profile, Smokers, Dyslipidemia, Karimnagar

INTRODUCTION

A large number of risk factor which predispose to atherosclerosis and Coronary Artery Disease (CAD) have been identified. These include modifiable ones like hypertension, dyslipidemia, smoking, diabetes mellitus, changing life style and non modifiable ones like age and sex. As the number of risk factor in an individual increases, so does the risk of developing atherosclerosis and its complication mainly coronary artery disease (CAD). In subject with more than one of these risk factor the risk is more additive.[1] Although smoking has been established as an independent risk factor for Coronary Heart Disease[2], the mechanism by which it increases the risk of coronary heart diseases is unclear. Four explanations have been postulated: the increased carbon monoxide in the blood of cigarette smokers may damage the endothelium and accelerate the entry of cholesterol into the wall of the artery[3]. The formation of carboxyhemoglobin creates relative anoxemia in the tissue, including the myocardium[4]. Smoking enhances platelets aggregation, and the nicotine absorbed from cigarette smoke may induce cardiac arrhythmia through its pharmacologic action.

An additional mechanism has been recently suggested that smoking adversely affect the concentration of the plasma lipids and lipoproteins. However, studies to date have revealed incomplete, inconclusive or conflicting results about the association of smoking on the plasma lipid and lipoprotein levels. In some studies, smokers had increased plasma cholesterol in other plasma cholesterol level have actually been lower only a few studies **ii**have specifically examined the plasma lipoprotein according to smoking status or number of cigarettes smoked (dosage) Smokers are reported to have higher low density lipoprotein (LDL) and lower high density cholesterol levels than non smokers. Finally conflicting data on plasma triglycerides levels in smokers have been reported.

There is an inadequate data on the association of smoking and dyslipidemia in India. Also there is wide spread habit of smoking cigarettes and beedies also increased prevalence of coronary artery disease among rural and urban population of India. Hence the present study has been taken up to find out the alteration of serum lipid profile (if any) between smokers &non smokers& also to see any dose related changes in serum lipid (if any) among the smoking population in Karimnagar.

SMOKING AND LIPID PROFILE

Smoking of tobacco by people started centuries ago but the health and environmental hazards, posed by it was recognized only in the 20th century. Atherogenesis, which is important risk factor for ischemic heart disease (IHD) and cerebrovascular accident, is thought to be accelerated by smoking. The exact atherogenic mechanism of smoking is still unclear. It has been observed, by workers that smoking lead to dyslipidemia which is a major factor for atherosclerosis.

The Framingham heart study was initiated in 1948 by the United States Public Health Service to study the relationship of number of risk factor (e.g. serum, cholesterol, blood pressure, weight smoking) to the subsequent development of cardiovascular disease. The town of Framingham (Massachusetts) had a population of 28,000 in 1948. The study was planned for 20 years in view of the slow development of heart disease[16].

The lower and upper limits of the study population, was set at 30 and 59 years of age. Out of 10,000 people, in this age group a sample of 6507 person of both sexes were invited to participate in the study, out of which 5209 participated. The initial examination revealed that 82 subject had clinically evident CHD. They were excluded from the sample leaving a total of 5127.

4469 (69%) of the 6507 in the initial sample actually underwent the first examination. After the first examination, the study population was examined every 2 year period. Information was obtained with regard to serum cholesterol, blood pressure, weight and cigarette smoking. Although biennial examinations were that main source of follow up information, other means were also adopted to detect CHD (e.g. Death Certificate Record)

Among other things, the study showed increasing risk of coronary heart disease (CHD) with increasing serum cholesterol level in the 45-54 age group. The study also showed that the association between smoking and CHD varied with manifestations of disease. Thus, smoking was more strongly associated with sudden death from CHD than with less fatal forms of the disease. Risk factor have been found to include male sex, advancing age, high serum lipids concentration, high blood pressure, cigarette smoking, diabetes mellitus, obesity, low vital capacity and certain ECG abnormalities. The predictive value of serum lipids, blood pressure and cigarette smoking had been repeatedly demonstrated. The Framingham heart study became a prototype of similar studies in US and other countries[16].

The relation between smoking and blood lipids and apolipoproteins (A1,B100) were studied in a group of 1024 12- to 18-year-old school children in the Comunidad de Madrid. The percentage of smokers was 19% (17% for girls and 21% for boys). The average consumption of cigarettes per day was 7.83 +/- 5.06 in boys and 6.04 +/-3.49 in girls (p less than 0.05). As compared with male nonsmokers, male smokers showed a higher mean level of low-density lipoprotein (LDL) cholesterol (112 versus 100 mg/dL, p less than 0.05), a higher LDL cholesterol to HDL-cholesterol ratio (2.27 versus 1.94, p less than 0.001), a higher mean level of apolipoprotein B100 (59 versus 53 mg/dL, p less than 0.05), and a higher apolipoprotein B100 to apolipoprotein A1 ratio (0.45 versus 0.40, p less than 0.01). Female smokers tended to show the same results, although significant differences were only found for LDL cholesterol to HDL cholesterol ratio and apolipoprotein B100 to apolipoprotein A1 ratio (1.8 versus 1.59 and 0.41 versus 0.38 respectively, both p less than 0.05). This work provides new data about the effects of smoking on apolipoproteins in adolescents and emphasizes on the need for preventive programs[17].

Development of coronary artery disease (Athersclerosis) begins in childhood itself (FELIC study). It is therefore important to identify potential risk factor early when prophylactic care must be cost effective. E.g. cigarette smoking[18].

The serum anti-atherogenic HDL-C level is significantly low in chronic smokers irrespective of the number of cigarettes smoked. The serum level of total cholesterol, LDL-C and VLDL-C and TG are significantly increased in persons smoking 11-20 cigarettes or beedis per day as compared to those smoking 1-10 cigarettes or beedis per day and therefore raising the cardiovascular disease risk[19].

Among non-smokers and light, Moderate, and heavy smokers a significant dose response effect was present for cholesterol (0, 1.8, 4.3, and 4.5% respectively, Triglycerides (0, 10.7, 11.5, and 18.0%), very low density lipoprotein cholesterol (0, 7.2, 44.4, and 39.0%), low density lipoprotein cholesterol (0, -1.1, 1.4, and 11.0%), high density lipoprotein cholesterol (0, -4.6, -6.3, and -8.9%), and apolipoprotein AI (0, -3.7 and -5.7% in non-smokers and light and heavy smokers).

These dose response effects may provide new evidence for a causal relation between exposure to cigarette smoke and changes in serum lipid and lipoprotein concentrations whether as a direct result of physiological changes or of

dietary changes induced by smoking. Adequate prospective data to estimate the excess risk of coronary artery disease existed only for cholesterol concentration. When that information was combined with data from the present study, and given that smokers as a group face an average overall excess risk of coronary artery disease of 70%, it was estimated that the observed increased serum cholesterol concentration in smokers may account for at least 9% of that excess risk. Furthermore, the dose response effect of smoking on serum cholesterol concentration suggests a gradient of increased absolute risk of coronary artery disease between light and heavy smokers[20].

The association between extent and duration of smoking habit and severity of coronary atheroma was examined in 387 patients undergoing routine coronary ateriography before valve replacement surgery. Total number of cigarettes smoked in life correlated significantly with severity of coronary artery disease ($p < 0\ 001$) and number of coronary arteries with stenoses of 50% or more ($p < 0\ 001$). Severity of coronary artery disease in current smokers was similar to that in former smokers. Multiple regression analysis showed diastolic blood pressure, cigarette consumption, age, ratio of total cholesterol to high density lipoprotein cholesterol, and history of angina to be the important predictors of severity of coronary artery disease. An estimate of the number of cigarettes smoked in life can be useful in identifying patients with coronary artery disease if used in conjunction with data on other important risk factors[21]

Smoking is the leading preventable cause of illness and premature death in Germany, claiming over 110,000 lives a year because it directly increases the risk of dying from heart disease, stroke, emphysema and a variety of cancers. The overwhelming majority of smokers begin tobacco use before they reach adulthood. Among those young people who smoke, the average age is now 13-14. In Germany, about 39% of male and 31% of female adults (age 18-60 years) continue to smoke, despite information about the unequivocally negative health consequences of smoking.

The exact mechanisms of smoking-related vascular disease are not yet known. Smoking causes acute hemodynamic alterations such as increase in heart rate, systematic and coronary vascular resistance, myocardial contractility, and myocardial oxygen demand. These short-term effects could lower the ischemic threshold in smokers with coronary artery disease and contribute to the increased risk for acute cardiovascular events. Endothelial damage is thought to be an initiating event in atherosclerosis and early studies have demonstrated that long-term smoking has direct toxic effects with structural changes of human endothelial cells. Recent research has shown the importance of the functional role of the endothelium in regulating vascular tone, platelet-endothelial interactions, leukocyte adhesion and smooth muscle cell proliferation via synthesis and release of a variety of substances such as nitric oxide.

There is strong evidence that smoking leads to endothelial dysfunction mainly by increased inactivation of nitric oxide by oxygen-derived free radicals. Smoking also increases oxidative modification of LDL and is associated with lower HDL plasma levels. Smoking induces a systemic inflammatory response with increased leukocyte count and elevation of the C-reactive protein level. Importantly, the prothrombotic effects of smoking have been repeatedly demonstrated to cause alterations in platelet function, imbalance of antithrombotic vsprothrombotic factors and decrease of fibrinolytic activity[22].

When compared with non-smokers, boy and girl smokers showed a significantly higher serum levels of total cholesterol, LDL-cholesterol, triglycerides and apolipoprotein B100, and significantly lower serum levels of HDL-cholesterol. Adolescent smokers tended to show a two-fold higher risk of altered lipid-lipoprotein levels than non-smokers[23].

Alcohol consumption was positively and linearly associated with high density lipoprotein cholesterol (HDL-C) levels and negatively associated with both low density lipoprotein cholesterol (LDL-C) levels and the ratio of total cholesterol (TC)/HDL-C ($P < 0.05$ to $P < 0.001$) among Japanese American males and Japanese American females and Native Japanese males. Current smoking habit was observed to be negatively associated with HDL-C levels and positively with TC/HDL-C ratio and log TG levels (logarithmic transformation of triglyceride values) ($P < 0.05$ to $P < 0.001$) among all three groups. Body mass index (BMI) was negatively associated with HDL-C levels and positively associated with log TG and TC/HDL-C ratio among all three groups ($P < 0.05$ to $P < 0.001$). Moderate alcohol consumption was negatively associated with log TG levels among Japanese American males and females ($P < 0.05$), whereas heavy alcohol consumption was positively associated with log TG levels in Native japanese males ($P < 0.001$). Smoking was positively associated with TC and LDL-C levels ($P < 0.05$) among Japanese American males, whereas a negative association ($P < 0.05$) was observed in Native Japanese males[24].

Thomas Heitzer and his associates demonstrate that cigarette smoking and hypercholesterolemia synergistically impair endothelial function and that their combined presence is associated with increased plasma levels of auto-antibodie against oxidized LDL. These observations raise the possibility that long-term smoking potentiates endothelial dysfunction in hypercholesterolemic patients by enhancing the oxidation of LDL[25].

Mean HDL cholesterol levels are lower in dyslipidemic children from households with smokers than in those without household smoke exposure. Passive smoking may worsen the risk profile for later atherosclerosis among high-risk young persons[26].

The prevalence of smoking among males and females was 33.2% (N=150) and 28.4% (N=108), respectively (mean cigarette consumption 13/day). As many as 349 males (77.2%) and 220 females (58.0%) reported consuming alcohol on a regular basis. The prevalence of low HDL-cholesterol (<0.9 mmol/l) was 14.5% in males and 5.1% in females, and of high LDL-cholesterol levels (>4.1 mmol/l) in 11.1% of male and 5.5% of female participants. Smoking was related to higher triglyceride (p=0.032), and lower HDL-cholesterol (p=0.037) serum levels. Total cholesterol, LDL-cholesterol, and the TC/HDL-cholesterol ratio were strongly related with the level of smoking (p=0.006, p=0.008, and p=0.006 respectively)[27].

Smokers in general and female smokers in particular had decreased alpha tocopherol levels when compared with non smokers. Smokers had low HDL cholesterol but this difference was statistically significant in females. Regardless of sex in smokers there was positive correlation between alpha tocopherol and triglyceride levels[28].

Kharb S, Singh GP studied changes in lipid peroxidation, vitamin E status and lipid profile due to smoking in healthy subjects, patients with acute myocardial infarction (MI), and in stabilized patients surviving MI. A significant increase in malondialdehyde (MDA) concentrations was observed in MI patients, more than in smokers (P<0.05), as compared to control. The plasma vitamins E as well as the ratio of vitamin E to lipids were significantly lower in MI patients as compared to stable ischemic heart disease (IHD) patients and controls. Our associated with lowered antioxidant status in MI.[29]

Tilwani R.K and associates who studies total cholesterol, triglycerides, LDL, VLDL and HDL found that TG, LDL, VLDL and TC were significantly high, in smokers when compared to non smokers. Increasing progressively from light to heavy smokers, showed a direct relationship and an inverse dose relationship was found inHDL in smokers[30].

A study of Khurana M and associates on lipid profile in cigarette smokers and tobacco chewers showed HDL cholesterol to be lower in smokers (p<0.001) as well as in tobacco chewers (p<0.001) than the controls. Both smokers and tobacco chewers had higher value of total cholesterol, low density lipoproteins cholesterol, very low density lipoproteins cholesterol and triglycerides as compared to non smokers, non tobacco chewers group. It was also noted smoking and tobacco chewing people have an equal and comparable adverse effect on lipid profile and therefore raisin cardiovascular risk[31].

Though different mode of addictions, smoking and tobacco chewing have equal and comparable adverse effects on lipid profile and therefore raising cardiovascular risk in same proportion.

In a survey of a healthy population (n = 197), LDL cholesterol, plasma triglycerides and VLDL triglycerides were found to be substantially increased and plasma HDL cholesterol decreased in smokers. The lipid-associated atherogenic risk in smokers as assessed by the LDL/HDL ratio was significantly higher [2.89 (SD 1.18, n = 63)] than in non-smokers [2.38 (SD 0.98, n = 86) P < 0.01]. The lower HDL level found in smokers was explained by a lower HDL-2 sub-fraction as determined by analytical ultracentrifugation. HDL 2b, 2a and 3a, measured by gradient gel electrophoresis, were all lower in the smokers but this was only significant for HDL 2a. Smoking had no effect on Lp(a) levels. HDL cholesterol and HDL-2 were strongly negatively correlated whereas LDL cholesterol and LDL/HDL ratio were strongly positively correlated with the plasma triglyceride concentration. There was a small but significant reduction in plasma CETP activity [non-smokers 49% t/micro liter (SD 17, n = 90), smokers 43% t/micro liter (SD 17, n = 66) P < 0.05] but CETP activity was not correlated with any measure of HDL in this population. Smoking was found to be an important independent contributor to the variation in plasma triglyceride, HDL, HDL-2 and LDL/HDL ratio. After correcting for sex, age, BMI, alcohol consumption, oral contraceptive use and plasma triglycerides smoking was still found to be significantly associated with HDL and the LDL/HDL ratio. Upon adjustment for covariant factors the mean differences between smokers and non-smokers for HDL cholesterol, HDL-2 and LDL/HDL were 0.15 mM, 16 mg dl-1 and 0.39 respectively. There appeared to be important sex differences in the influence of smoking on plasma lipoproteins. In women the main impact of smoking was on triglyceride levels and they in turn affected LDL and HDL. In contrast, in men, smoking had little impact on triglycerides and affected HDL more directly. We conclude that smoking cigarettes has an important effect on plasma lipoprotein metabolism through multiple mechanisms[32].

Tiwari A.K and his associates studied the effect of cigarette smoking on serum total cholesterol and HDL cholesterol in normal subjects and coronary heart patients. 51 normal volunteers and 34 clinically established

coronary heart disease patients were studied. 21 out of 51 normal and 16 out of 34 coronary heart disease were cigarette smokers. The cases were divided into two groups aged 20-40 years the younger age group and 41-61 years the older age group. TC and HDL cholesterol of all causes where determined. The ratio of total cholesterol to HDL cholesterol was significantly higher in all normal and coronary heart diseases smokers. Hence, the higher level of total cholesterol to high density lipoprotein cholesterol ratio approved toxic, one of the important parameter helps to ascertain the development of coronary heart diseases in cigarette smokers[33]. Where as mean HDL cholesterol (43.2±5.8mg/dL) was significantly lower ($p<0.05$). Mean triglycerides (170.8±59.7mg/dL) was significantly higher in smokers than non smokers ($p<0.01$) in the fed state the total cholesterol level and triglyceride levels was increased by 10.4mg/dL and 51.1mg/dL respectivly in smokers where as the increase was 4.8mg/dL and 24.3mg/dL respectively in non smokers. There was less rise of HDL cholesterol (1.9mg/dL) in smokers as compared to non smokers 3.4mg/dL in feed states[34].

high-density lipoprotein phospholipid and in apolipoprotein AI concentration (p less than 0.01), whereas high-density lipoprotein triglyceride concentrations did not change significantly. These findings confirm and extend those of earlier cross-sectional studies which showed low concentrations of high-density lipoproteins in cigarette smokers, A significant correlation between the rise in high-density lipoprotein cholesterol concentrations and the increase in fat consumption after stopping smoking indicate that the changes in high-density lipoprotein concentrations may be partly due to nutritional factors[35].

In smokers and nonsmokers, respectively, the mean (+/-SD) lag times of diene formation were 111 +/- 26 and 100 +/- 27 min, the peak rates of diene formation (Vmax) were 5.99 +/- 2.34 and 6.34 +/- 2.30 mmol x min-1 x g-1, and the amounts of dienes produced during the propagation phase (dmax) were 250 +/- 264 and 248 +/-56 mmol x g-1. Neither the malondialdehyde content of LDL (measured as thiobarbituric acid-reactive substances) before oxidation nor the amount of malondialdehyde generated during oxidation (smokers: 57.0 +/- 14.2 micromol x g-1; nonsmokers: 63.2 +/- 15.2 micromol x g-1) indicated any statistically significant effect of smoking. When nonsmokers and smokers were considered together, the amount of malondialdehyde generated during oxidation correlated with age (nonparametric rs =0.405), body mass index (r2 = 0.573), and concentrations of apo B (0.480), cholesterol (rs = 0.448), triglycerides (rs = 0.436), and LDL cholesterol (rs = 0.398). Our data show that smoking is not associated with increased oxidizability of LDL in healthy men and women at ages 42-63 years[36].

Lipoproteins

Lipoproteins are macromolecular complexes that carry hydrophobic plasma lipids, particularly cholesterol and triglyceride, in the plasma. They transport essential fatty acids and all other cholesterols and esterified lipids in blood.

Type of plasma lipoproteins

Four major classes and two minor classes of plasma lipoproteins are identified based on particle size, based on size, chemical characteristic, floctation characteristic and electrophoretic mobility.

Four major lipoproteins Chylomicrons, Very low density Lipoprotein (VLDL), Low density Lipoprotein (LDL), High density Lipoproteins (HDL).

Two minor lipoproteins Intermediate Density Lipoproteins (IDL) , Lipoprotein Little (a)

The protein moiety of lipoprotein is composed of several specified proteins called as apolipoproteins. VLDL account for most of the triglycerides in the plasma, LDL carries most of the cholesterol in normal plasma. The LDL is about 50% by weight cholesterol and 20% by weight protein. HDL are about 50% by weight protein and 50% by weight lipid.

Very Low Density Lipoprotein (VLDL)

VLDL is major transport vehicle for endogenously synthesized and predominantly synthesized in liver. VLDL particle are smaller than chylomicrons and are rich in triglycerides they have a lower lipids / proteins ratio thus float at a somewhat higher density. When excessive amount of VLDL are present the plasma appear turbid. VLDL triglyceride which are of endogenous origin, mainly hepatic and about half the particles mass. VLDL particle size varies widely with concomitant variation of chemical composition. Smaller particle depleted of triglyceride and surface material result from hydrolysis of VLDL by lipoprotein lipase. These particle are referred to as VLDL remanants and intermediate density lipoprotein (IDL).

Low density lipoprotein (LDL)

LDL is the principal vehicle for the transport of cholesterol from liver to body cells. LDL is formed in the circulation by progressive removal of triglyceride from VLDL. LDL consists of about 50% of total lipoprotein mass in human plasma. LDL particles are much smaller than the triglycerides rich lipoproteins. Cholesterol most of which is esterified account for about half of the LDL mass. About 25% of LDL mass is protein moiety. (apo-100, with trace of apo-c) Discrete sub fractions of LDL have been identified that differ somewhat in their size and chemical composition. The smaller species of LDL contain lower amounts of cholesterol ester resulting in lower ratio of cholesterol to Apo B in these particles than in larger species of LDL. Increased amount of smaller particles have been with several common form of dyslipoprteinemia that are associated with Coronary Artery diseases (CAD).

Synthesis and high dose may be used for hypertriglyceridemia. When the triglyceride levels of >500 mg/dl are generally treated with drugs, whereas lower level (200-500 gm/dl) are not treated unless other CHD risk factors are present.

More intensive treatment is more effective in producing long-term abstinence from tobacco. Nicotine replacement therapy (nicotine patches or gum), clinician-delivered social support, and skills training are the three most effective components of smoking cessation treatment. A framework for tobacco control measures is necessary to reduce tobacco consumption and exposure to tobacco smoke.

SMOKING AND CORONARY HEART DISEASES

Coronary heart disease is common in both the developed countries and developing countries. It is estimated that approximately 1.5 million infarcts occur in USA alone. Hyperlipidemia a major risk factor for atherosclerosis is characterized by raised level of lipids (Triglycerides cholesterol) and lipoproteins (low density lipoproteins) and very low density lipoproteins. These factors vary among various socioeconomic states [41].

Cholesterol was postulates to be related to atherosclerosis when it was found to be a major component of advanced atherosclerotic plaques. The association between elevated serum cholesterol and atherosclerotic diseases was first reported in 1903, subsequently large epidemiologically studies such as the seven countries study and Framingham heart study confirmed relationship between serum cholesterol and CAD in the multiple risk factor intervention trial (MRFIT), the relationship between serum cholesterol level and subsequent CAD was found to be continuous, graded and strong. The lipid research clinics prevalence study demonstrated in a 10 years follow up that low density lipoprotein cholesterol was strongly associated with CAD death in men with or without CAD[42].

Cigarette smoking is the most preventable cause of cardiovascular morbidity and mortality. Smoking has been associated with a two-to fourfold increased risk of coronary heart disease, a greater than 70% excess rate of death from coronary heart disease, and an elevated risk of sudden death. These risks are compounded in the presence of hypertension, hypercholesterolemia, glucose intolerance, and diabetes, all of which exhibit a synergistic effect with smoking. The relationship between smoking and the risk of peripheral vascular disease has also been well documented. Smokers account for approximately 70% of patients with atherosclerosis obliterans and virtually all those with thromboangiitisobliterans. An association between smoking and cerebrovascular disease remains a matter of debate, although a higher risk of stoke and stroke-related mortality has been observed in smokers than in nonsmokers. Smoking has also been implicated in the development of corpulmonale, but a direct association with congestive heart failure has not been established. Nicotine and carbon monoxide appear to play major roles in the cardiovascular effects of smoking. Both components adversely alter the myocardial oxygen supply/demand ratio and have been shown to produce endothelial injury, leading to the development of atherosclerotic plaque. Adverse effects on the lipid profile have been noted as well, but the relationship between these changes and the risk of cardiovascular disease remains to be confirmed. Notably, smoking cessation results in a dramatic reduction in the risk of mortality from both coronary heart disease and stroke. In light of the fact that the incidence of smoking has declined primarily among educated sectors of the U.S. population, future efforts must focus on providing effective education, including smoking cessation techniques, to the less-educated groups[43].

Smoking is a major risk factor for atherosclerosis and coronary heart disease, cigarette smoking acts both independently and synergistically with other risk factor like hypertension and hypercholesterolemia. Mortality from coronary heart disease is substantially higher in cigarette smokers than non smokers. It is generally believed that harmful cardiovascular effects of smoking are partly caused by changes in lipid metabolism.

Nine hundred and seventy eight patients with a first documented myocardial infarction were studied to detect smoking related differences in clinical profile and in-hospital outcome. The distribution of infarct sites differed significantly between smokers and non-smokers. Smokers had higher peak cardiac enzyme concentrations. In spite of this, smokers had a better prognosis than non-smokers. There are important differences between smokers and

non-smokers, both in clinical profile and in-hospital outcome, which may reflect a difference in the nature of the underlying coronary disease. Smoking is a major risk factor for coronary heart disease. Although the long term prognosis after a first myocardial infarction has been compared in smokers, non-smokers, and ex-smokers,' the relation of smoking status to the manifestations of acute coronary heart disease remains unclear. The purpose of this study was to examine the extent to which smokers, non-smokers, and ex-smokers show marked differences in clinical profile and in-hospital outcome after a first myocardial infarction[45].

Cigarette smoking is a main risk-factor for enhanced cardiovascular morbidity and mortality. Some studies have even suggested that involuntary smoking increases the atherosclerotic risk. Smoking related cardiovascular diseases include coronary heart disease, acute myocardial infarction, sudden death, stroke, aortic aneurysm, atherosclerotic peripheral vascular disease. Risk is potentiated in patients with other coronary risk-factors i.e. hypertension and/or hypercholesterolemia. It is also proportionately related to the number of cigarettes smoked daily and smoking behavior. Combination of cigarette smoking and oral contraceptive use is the major cause of coronary events in female smokers under 50 years. Risk will be reduced only be smoking cessation. Underlying pathophysiologic mechanisms are complex; nicotine- and carbon-monoxide induced deleterious effects will be found on hemodynamic parameters, lipid status and hemorheology. Although clinical events due to acute coronary thrombosis and vasoconstriction are more often in smokers than in nonsmokers, angina pectoris is less common. Furthermore smoking diminishes beneficial effects of well established therapeutical procedures in treatment of coronary heart disease. Therefore, smoking cessation therapy should be a major goal for primary and secondary prevention programs as well[46]. Smoking as a cardiovascular risk factor and the clinical cardiovascular features associated with active and passive smoking are discussed, and a pathophysiological framework to explain the association between cigarette smoking and cardiovascular disease is provided[47].

Both active smoking and Environmental Tobacco Smoke exposure are associated with the progression of an index of atherosclerosis. Smoking is of particular concern for patients with diabetes and hypertension. The fact that pack-years of smoking but not current versus past smoking was associated with progression of atherosclerosis suggests that some adverse effects of smoking may be cumulative and irreversible [48].

Breathing other people's smoke is an important and avoidable cause of ischaemic heart disease, increasing a person's risk by a quarter [49]. Smoking is a major cause of coronary heart disease for both men and women and a positive correlation between tobacco use and cerebrovascular disease has been also described. In addition, cigarette smoking is the most powerful risk factor predisposing to atherosclerotic peripheral artery disease. More recently, passive smoking has been also shown to represent an important risk factor for coronary artery disease. Moreover, the incidence of coronary artery and cerebrovascular diseases in ex-smokers consistently decreases after cessation, further underlying the relevance of smoking as a risk factor for this pathological condition[50]. CS increases inflammation, thrombosis, and oxidation of low-density lipoprotein cholesterol. Recent experimental and clinical data support the hypothesis that cigarette smoke exposure increases oxidative stress as a potential mechanism for initiating cardiovascular dysfunction[51].

EXPERIMENTAL SECTION

Major classes of human lipoprotein and their physiochemical characteristics

Table No. 1 Major Lipoprotein classes and their Chemical Compositions (% of dry Mass)

Lipoprotein	Density Gm/ml	Diameter Nm	Electrophoretic Mobility	SF
Chylomicrons	<0.96	800-500	Alpha – 2	>400
VLDL	0.96 – 1.006	300-800	Pre – B	20-200
IDL	1.006-1.019	250-350	Slow pre-B	12-20
LDL	1.019-1.063	216	B	0-12
HDL 2	1.063-1.125	100	Alpha – 1	-
HDL 3	1.125-1210	75	Alpha _ 1	-
LP(a)	1.055-1.085	300	Slow pre- B	-

Table No. 2 NCEP Guidelines for Classification of Lipid Profile[38]

Lipoprotein	Triglycerides	Cholesterol Esters	Cholesterol	Phospholipids	Proteins
Chylmicrons	86	3	2	7	2
VLDL	55	12	7	18	8
IDL	23	29	9	19	19
LDL	6	42	8	22	22
HDL 2	5	17	5	33	40
HDL 3	3	13	4	25	55
LP (a)	3	13	9	22	33

Table No. 3.

Lipids constituent	Desirable (mg/dl)	Borderline to High (mg/dl)	High (mg/dl)
Total cholesterol	<200	200-239	>240
LDL cholesterol	<130	130-159	>160
Triglycerides	<150	150-499	>500
HDL cholesterol	>60	40-59	<40

In hyperlipidemia one or more classes of lipoproteins may accumulate in the blood as a result of either their increased production or secretion into circulation or their decreased clearance from the circulation or both.

Alternations resulting from genetic defect are classified as primary disorders of lipid metabolism. Alternation associated with certain known condition like diabetes mellitus, hypothyroidism, nephritic syndrome are classified as secondary disorders of lipid metabolism.

Fredrickson classification of hyperlipidemia API[39]

Table No. 4

Phenotype	**Lipoprotein Elevated**	**Plasma cholesterol Level**	**Plasma triglycerides level**	**Atherogenecity**	**Reflective Frequency**
1a	Chylomicrons	Normal to		Not known	<1%
2a	LDL		normal	Very high	10%
2b	LDL,VLDL			Very high	40%
3	LDL			Very high	<1%
4	VLDL	Normal to		High	45%
5	VLDL& chylomicrons	To		Higher	5%

↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑

Mildly raised
↑
Moderately raised
↑↑
Severally
↑↑↑
Very Severally
↑↑↑↑

Lipid Lipoprotein Normograms in Indian Population and Recommended Level[39] (Mean Value in mg/dL)

Table No. 5

	TG	**Cholesterol**	**HDLc**	**LDLc**
East	115	185	42	115
South	155	180	38	107
	119	172	40	108
West	107	188	38	129
North	132	150	43	101
Recommended	≤ 150	≤ 200	≥ 40	≤ 130

Treatment decision based on LDL cholesterol Level of LDL for beginning therapy (mg/dl)

Table No. 6

Assessment	Diet	Drugs	Goal
No CHD and less	≥160	≥190	<160
Then 2 risk factors			
No CHD but 2 or	≥130	≥160	<130
more Risk factors			
Presence of CHD	>100	>130	<100

A. ESTIMATION OF PLASMA TOTAL CHOLESTEROL[52]

The total cholesterol was estimated by the enzymatic method (cholesterol Oxidase –Peroxidase) with Endpoint Colorimetry.

Principle: The estimation of cholesterol involves the following enzymatic reactions: CE

Cholesterol esters	Cholesterol + Fatty acids
	CO
Cholesterol + O_2	Choleste-3-one + H_2O_2
	POD
H_2O_2 + Phenol + 4-AAP	Quinoneimine dye + H_2O

CE = Cholesterol esterase, CO = Cholesterol Oxidase, POD = Peroxidase, 4AAP = 4-Aminoantipyrine.

Absorbance of quinoneimine measured at 505 nm is proportional to cholesterol concentration in the specimen.

Reagents:
Cholesterol Mono reagent: Goods Buffer (pH 6.7) ……. 50 mmol / L Cholesterol Oxidase ……...≥ 50 U / L Cholesterol Esterase……≥ 100 U/ L Peroxidase…….≥ 3 KU /L Chromogen Stabilizers

Reagent 2 Cholesterol Standard Cholesterol standard = 200 mg /dL Preservative stabilizer

Sample collection: Un-hemolized sample of serum or plasma collected in heparin or EDTA may also be used.

Equipment: Semi automated analyzer (ERBA, Transasia Ltd)

Assay Program

Mode	End Point
Wavelength	505nm (490 – 530)
Temperature	37^0C
Optical path length	1 cm
Blanking	Reagent blank
Sample volume	10µL
Working reagent volume	1000µL
Incubation time (mins)	10 at 37^0C
Concentration of Standard	200 mg / dL
Maximum absorbance limit	2.0
Linearity	750 mg / dL
Stability of color	1 hour
Units	mg / dL

Procedure:

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Serum / Plasma	--------	----------	10µL
Standard	--------	10µL	--------
Cholesterol Reagent	1000µL	1000µL	1000µL

• **ESTIMATION OF PLASMA TRIGLYCERIDES**[53]

Plasma triglycerides were estimated by enzymatic (Glycerol-3-phosphate Oxidase) method with Endpoint colorimetry.

Principle: The estimation of Triglycerides involves the following enzymatic reactions LPL

⟶ Triglycerides +	Glycerol + FFA
GK	
Glycerol + ATP	Glycerol-3-Phosphate + ADP
	GPO
Glycerol-3-Phosphate + O_2	DHAP + H_2O_2
POD	
$2H_2O_2$ + 4- AAP	Quinoneimine dye + $4H_2O$

⟶ ⟶ ⟶

LPL = Lipoprotein Lipase; FFA = Free Fatty Acids; GK = Glycerol Kinase, GPO =Glycerol –3– Phosphate Oxidase; POD = Peroxidase, ATP = Adenosine Triphosphate ; AAP = 4- Aminoantipyrine ; ADP = Adenosine Diphosphate; DHAP = Dihydroxyacetone phosphate.

Absorbance of quinoneimine measured at 505 nm is proportional to Triglycerides concentration in the specimen.

Reagents

Reagent 1 Triglycerides Mono Reagent:

Pipes Buffer …….. 50 mmol / L 4-Chlorophenol…….. 05 mmol / L Magnesium ion…….. 05 mmol / L ATP…… 1.0 mmol / L Lipase……. ≥ 5000 U / L Peroxidase…….. ≥ 1000 U / L Glycerol Kinase …… ≥ 400 U / L 4-Aminoantipyrine…… 0.4 mmol U / L Glycerol – 3 – Phosphate Oxidase….. ≥ 4000 u / l Detergents, Preservative & stabilizer

Reagent 1 Triglycerides Standards: Triglycerides standard 200 mg / dL
Sample:Unhemolized sample of serum or plasma collected in heparin or EDTA may also be used.
Equipment: Semi automated analyzer (ERBA, Transasia Ltd)

Procedure

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Serum / Plasma	--------	----------	10µL
Standard	--------	10µL	--------
Triglycerides Reagent	1000µL	**ii**1000µL	1000µL

Assay Programme

Mode	**End Point**
Wavelength	510 nm (505-530)
Temperature	25-30^{0}C
Optical path length	1 cm
Blanking	Reagent blank
Sample volume	50µL
Working reagent volume	100µL
Incubation time (mins)	10 at 37^{0}C
Concentration of Standard	50 mg / dL
Maximum absorbance limit	2.0
Linearity	400 mg / dL
Stability of color	2 hour
Units	mg / dL

Treatment decision based on LDL cholesterol
Level of LDL for beginning therapy (mg/dl)

Table No. 6

Assessment	Diet	Drugs	Goal
No CHD and less	≥160	≥190	<160
Then 2 risk factors			
No CHD but 2 or	≥130	≥160	<130
more Risk factors			
Presence of CHD	>100	>130	<100

Distribution of smokers based on the number of cigarettes or beedies smoke per day.

Table No. 7

Group	No. of Subjects	Percentage
Mild Smokers :	21	42%
1 – 10 Cigarettes		
1-15 beedies per day		
Moderate smokers	16	32%
11-20 cigarettes or		
16-30 beedies per day		
Heavy smokers :	13	26%
> 20 cigarettes or		
> 30 beedies per day		
Total	**50**	**100%**

Comparison of lipid profile in smokers and non smokers

Table No. 8

Lipid Profile	Smoker	Control	P
TG	169.4±53.43	130.16±35.58	<0.0001
TC	200.44±33.47	170.48±29.18	<0.0001
HDL	34.74±6.23	41.22±3.33	<0.0001
LDL	132.94±34.56	104.36±27.05	<0.0001
VLDL	32.58±10.37	25.96±7.04	0.0000

Values are mean ± standard deviation in mg/dl
P values are derived from analysis of variants

Age wise comparison of TG among smokers and non smokers

Table No. 9

Age		TG			
		No. of Subjects	Smoker	No. of Subjects	Control
15	19	3	196.66	3	121.67
20	24	18	169.61	15	130.94
25	29	16	146.67	16	128.88
30	35	13	161.30	16	132.32

Age wise comparison of TC among smokers and non smokers with respect to age

Table No. 10

Age		TC			
		No. of Subjects	Smoker	No. of Subjects	Control
15	19	3	201.67	3	191.67
20	24	18	192.67	15	173.20
25	29	16	178.10	16	170.94
30	35	13	193.47	16	163.50

Comparison of HDL among smokers and non smokers with respect to age

Table No. 11

Age		HDL			
		No. of Subjects	Smoker	No. of Subjects	Control
15	19	3	34.34	3	39.34
20	24	18	36.45	15	42.94
25	29	16	35.39	16	40.38
30	35	13	33.08	16	40.815

Comparison of LDL among smokers as compared to non smokers with respect to age

Table No.12

Age		LDL			
		No. of Subjects	Smoker	No. of Subjects	Control
15	19	3	124.00	3	79.34
20	24	18	132.23	15	103.47
25	29	16	118.93	16	104.50
30	35	13	139.92	16	109.80

SUMMARY AND CONCLUSION

Smoking causes various complications including COPD, Lung cancer and Dyslipidemia. Dyslipidemia in turn causes Ischemic Heart disease. 50 male smoker between the age of 15 to 35 years and 50 male non smokers between the age o f 15 to 35 years without any other cause for dyslipidemia as control were selected for this study. Fasting blood samples from all smokers and non smokers were collected and serum lipid profile was essayed. Results indicated a significant decrease in serum HDL which was seen in 74% of the studied subjects (HDL < 40 mg/dL) and increases in TG, TC and LDL levels which was seen in 58%, 50% and 58% respectively the cutoff value being TG > 150 mg/dL, TC > 200 mg/dL and LDL > 130 mg/dL. Direct relation exists between severity and duration of smoking with an increase in total cholesterol TG and LDL. HDL showed an inverse relationship. The alteration of lipid profile in smokers have raised serious medical concern with respect to Atherogenic Risk and recommendation for counseling the smokers to quit smoking and routine evaluation of serum lipid profile has been suggested.

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